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Spratt, Sharon Kaye  
Sangamo Biosciences, Inc.

<120> Regulation of Endogenous Gene Expression in Cells Using Zinc Finger Proteins

<130> 019496-002200US

<140> 09/229,037

<141> 1999-01-12

<160> 40

<170> PatentIn Ver. 2.0

 $\langle 210 \rangle$  1

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<220>  
<223> Description of Artificial Sequence:exemplary motif  
of C2H2 class of zinc finger proteins (ZFP)

<220>

<221> MOD RES

$\langle 222 \rangle$  (2) . . (3)

<223> Xaa = any amino acid

 $\langle 220 \rangle$ 

<221> MOD RES

$\langle 222 \rangle$  (4) . . (5)

<222> (4)..(5)  
<223> Xaa = any amino acid, may be present or absent

 $\langle 220 \rangle$ 

<221> MOD\_RES

 $\langle 222 \rangle \quad (7) \dots (18)$ 

<223> Xaa = any amino acid

 $\langle 220 \rangle$ 

<221> MOD\_RES

 $\langle 222 \rangle \quad (20) \dots (22)$ 

<223> Xaa = any amino acid

 $\langle 220 \rangle$ 

<221> MOD\_RES

<222> (23) .. (24)

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<222> (23)..(24)
<223> Xaa = any amino acid, may be present or absent
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<400> 1

<400> 1  
Cys Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
5 10 15

1

5

10

15

Xaa Xaa His Xaa Xaa Xaa Xaa Xaa His  
20 25

<210> 2  
 <211> 10  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:ZFP target site  
 with two overlapping D-able subsites

<220>  
 <221> modified\_base  
 <222> (1)..(2)  
 <223> n = g,a,c or t

<220>  
 <221> modified\_base  
 <222> (5)  
 <223> n = g,a,c or t

<220>  
 <221> modified\_base  
 <222> (8)  
 <223> n = g,a,c or t

<220>  
 <221> modified\_base  
 <222> (9)  
 <223> n = a,c or t; if g, then position 10 cannot be g  
 or t

<220>  
 <221> modified\_base  
 <222> (10)  
 <223> n = a or c; if g or t, then position 9 cannot be g

<400> 2  
 nngkngknnn

10

<210> 3  
 <211> 10  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:ZFP target site  
 with three overlapping D-able subsites

<220>  
 <221> modified\_base  
 <222> (1)..(2)  
 <223> n = g,a,c or t

<220>  
 <221> modified\_base  
 <222> (5)  
 <223> n = g,a,c or t

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<220>  
 <221> modified\_base  
 <222> (8)  
 <223> n = g,a,c or t

<400> 3  
 nngkngkngk

10

<210> 4  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:linker

<400> 4  
 Asp Gly Gly Gly Ser  
 1 5

<210> 5  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:linker

<400> 5  
 Thr Gly Glu Lys Pro  
 1 5

<210> 6  
 <211> 9  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:linker

<400> 6  
 Leu Arg Gln Lys Asp Gly Glu Arg Pro  
 1 5

<210> 7  
 <211> 4  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:linker

<400> 7  
 Gly Gly Arg Arg  
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<210> 8  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:linker

<400> 8  
 Gly Gly Gly Gly Ser  
     1                    5

<210> 9  
 <211> 8  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:linker

<400> 9  
 Gly Gly Arg Arg Gly Gly Gly Ser  
     1                    5

<210> 10  
 <211> 9  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:linker

<400> 10  
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     1                    5

<210> 11  
 <211> 12  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:linker

<400> 11  
 Leu Arg Gln Lys Asp Gly Gly Gly Ser Glu Arg Pro  
     1                    5                    10

<210> 12  
 <211> 16  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:linker

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<400> 12  
 Leu Arg Gln Lys Asp Gly Gly Gly Ser Gly Gly Gly Ser Glu Arg Pro  
           1                  5                  10                  15

<210> 13  
 <211> 25  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:ZFP target site  
           region surrounding initiation site of vascular  
           endothelial growth factor (VEGF) gene containing  
           two 9-base pair target sites

<220>  
 <221> protein\_bind  
 <222> (4)..(12)  
 <223> upstream 9-base pair ZFP VEGF1 target site

<220>  
 <221> protein\_bind  
 <222> (14)..(22)  
 <223> downstream 9-base pair ZFP VEGF3a target site

<400> 13  
 agcggggagg atcgcgagg cttgg

25

<210> 14  
 <211> 298  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:VEGF1 ZFP  
           construct targeting upstream 9-base pair target  
           site in VEGF promoter

<220>  
 <221> CDS  
 <222> (2)..(298)  
 <223> VEGF1

<400> 14  
 g gta ccc ata cct ggc aag aag aag cag cac atc tgc cac atc cag ggc 49  
 Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly  
           1                  5                  10                  15

tgt ggt aaa gtt tac ggc aca acc tca aat ctg cgt cgt cac ctg cgc 97  
 Cys Gly Lys Val Tyr Gly Thr Thr Ser Asn Leu Arg Arg His Leu Arg  
                   20                  25                  30

tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt ggt 145  
 Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly  
                   35                  40                  45

aaa cgc ttc acc cgt tgc tca aac ctg cag cgt cac aag cgt acc cac 193  
 Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His  
           50                  55                  60

T02020"14345850

acc ggt gag aag aaa ttt gct tgc ccg gag tgt ccg aag cgc ttc atg 241  
 Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met  
 65 70 75 80

cgt agt gac cac ctg tcc cgt cac atc aag acc cac cag aat aag aag 289  
 Arg Ser Asp His Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys  
 85 90 95

ggt gga tcc  
 Gly Gly Ser

298

<210> 15  
 <211> 99  
 <212> PRT  
 <213> Artificial Sequence

<400> 15  
 Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly  
 1 5 10 15

Cys Gly Lys Val Tyr Gly Thr Thr Ser Asn Leu Arg Arg His Leu Arg  
 20 25 30

Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly  
 35 40 45

Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His  
 50 55 60

Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met  
 65 70 75 80

Arg Ser Asp His Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys  
 85 90 95

Gly Gly Ser

<210> 16  
 <211> 298  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: VEGF3a ZFP  
 construct targeting downstream 9-base pair target  
 site in VEGF promoter

<220>  
 <221> CDS  
 <222> (2)..(298)  
 <223> VEGF3a

<400> 16  
 g gta ccc ata cct ggc aag aag aag cag cac atc tgc cac atc cag ggc 49  
 Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly  
 1 5 10 15

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tgt ggt aaa gtt tac ggc cag tcc tcc gac ctg cag cgt cac ctg cgc 97  
 Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg  
                   20                                  25                                  30

tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt ggt 145  
 Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly  
                   35                                  40                                  45

aaa cgc ttc acc cgt tgc tca aac cta cag agg cac aag cgt aca cac 193  
 Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His  
                   50                                  55                                  60

acc ggt gag aag aaa ttt gct tgc ccg gag tgt ccg aag cgc ttc atg 241  
 Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met  
                   65                                  70                                  75                                  80

cga agt gac gag ctg tca cga cat atc aag acc cac cag aac aag aag 289  
 Arg Ser Asp Glu Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys  
                                   85                                  90                                  95

ggt gga tcc  
 Gly Gly Ser

<210> 17  
 <211> 99  
 <212> PRT  
 <213> Artificial Sequence

<400> 17  
 Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly  
                   1                                  5                                  10                                  15

Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg  
                   20                                  25                                  30

Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly  
                   35                                  40                                  45

Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His  
                   50                                  55                                  60

Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met  
                   65                                  70                                  75                                  80

Arg Ser Asp Glu Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys  
                                   85                                  90                                  95

Gly Gly Ser

<210> 18  
 <211> 29  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: VEGF DNA target  
           site 1 recognition (top) strand

TO2020"44326860

<220>  
 <221> protein\_bind  
 <222> (11)..(19)  
 <223> VEGF DNA ZFP target site 1

<400> 18  
 catgcatagc ggggaggatc gccatcgat

29

<210> 19  
 <211> 29  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:VEGF DNA site 1  
 complementary (bottom) strand

<400> 19  
 atcgatggcg atcctccccg ctatgcatg

29

<210> 20  
 <211> 29  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:VEGF DNA  
 target site 3 recognition (top) strand

<220>  
 <221> protein\_bind  
 <222> (11)..(19)  
 <223> VEGF DNA ZFP target site 3

<400> 20  
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29

<210> 21  
 <211> 29  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:VEGF DNA target  
 site 3 complementary (bottom) strand

<400> 21  
 atcgatgcca agcctccgag atatgcatg

29

<210> 22  
 <211> 29  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:primer SPE7

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29

26

28

26

83

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<210> 27
<211> 39
<212> DNA
<213> Artificial Sequence
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<220>  
 <223> Description of Artificial Sequence:primer GB19

<400> 27 39  
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<210> 28  
 <211> 33  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:primer GB10

<400> 28 33  
 cagatcggat ccacccttct tattctggtg ggt

<210> 29  
 <211> 589  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:designed  
 6-finger ZFP VEGF3a/1 from KpnI to BamHI

<220>  
 <221> CDS  
 <222> (2)..(589)  
 <223> VEGF3a/1

<400> 29  
 g gta ccc ata cct ggc aag aag aag cag cac atc tgc cac atc cag ggc 49  
 Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly  
 1 5 10 15

tgt ggt aaa gtt tac ggc cag tcc tcc gac ctg cag cgt cac ctg cgc 97  
 Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg  
 20 25 30

tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt ggt 145  
 Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly  
 35 40 45

aaa cgc ttc aca cgt tcg tca aac cta cag agg cac aag cgt aca cac 193  
 Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His  
 50 55 60

aca ggt gag aag aaa ttt gct tgc ccg gag tgt ccg aag cgc ttc atg 241  
 Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met  
 65 70 75 80

cga agt gac gag ctg tct aga cac atc aaa acc cac cag aac aag aaa 289  
 Arg Ser Asp Glu Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys  
 85 90 95

gac ggc ggt ggc agc ggc aaa aag aaa cag cac ata tgt cac atc caa 337  
 Asp Gly Gly Gly Ser Gly Lys Lys Lys Gln His Ile Cys His Ile Gln  
 100 105 110

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ggc tgt ggt aaa gtt tac ggc aca acc tca aat ctg cgt cgt cac ctg 385  
 Gly Cys Gly Lys Val Tyr Gly Thr Thr Ser Asn Leu Arg Arg His Leu  
 115 120 125

cgc tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt 433  
 Arg Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys  
 130 135 140

ggt aaa cgc ttc acc cgt tcg tca aac ctg cag cgt cac aag cgt acc 481  
 Gly Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr  
 145 150 155 160

cac acc ggt gag aag aaa ttt gct tgc ccg gag tgt ccg aag cgc ttc 529  
 His Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe  
 165 170 175

atg cgt agt gac cac ctg tcc cgt cac atc aag acc cac cag aat aag 577  
 Met Arg Ser Asp His Leu Ser Arg His Ile Lys Thr His Gln Asn Lys  
 180 185 190

aag ggt gga tcc 589  
 Lys Gly Gly Ser  
 195

<210> 30  
 <211> 196  
 <212> PRT  
 <213> Artificial Sequence

<400> 30  
 Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly  
 1 5 10 15

Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg  
 20 25 30

Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly  
 35 40 45

Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His  
 50 55 60

Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met  
 65 70 75 80

Arg Ser Asp Glu Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys  
 85 90 95

Asp Gly Gly Gly Ser Gly Lys Lys Lys Gln His Ile Cys His Ile Gln  
 100 105 110

Gly Cys Gly Lys Val Tyr Gly Thr Thr Ser Asn Leu Arg Arg His Leu  
 115 120 125

Arg Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys  
 130 135 140

Gly Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr  
 145 150 155 160

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His Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe  
 165 170 175

Met Arg Ser Asp His Leu Ser Arg His Ile Lys Thr His Gln Asn Lys  
 180 185 190

Lys Gly Gly Ser  
 195

<210> 31  
 <211> 42  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:JVF9 VEGF3a/1  
 target oligonucleotide

<400> 31  
 agcgagcggg gaggatcgcg gaggcttggg gcagccgggt ag 42

<210> 32  
 <211> 42  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:JVF10 VEGF3a/1  
 target oligonucleotide complementary sequence

<400> 32  
 cgctctaccc ggctgcccc a gcctccg cg atcctccccg ct 42

<210> 33  
 <211> 25  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:primer JVF24

<400> 33  
 cgcggatccg cccccccgac cgatg 25

<210> 34  
 <211> 62  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:downstream  
 primer JVF25

<400> 34  
 ccgcaagctt acttgatc gtcgtccttg tagtcgtgc cccaccgta ctcgtcaatt 60

cc

62

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 102020-44826860

<210> 35  
 <211> 7  
 <212> PRT  
 <213> Simian virus 40

<220>  
 <221> PEPTIDE  
 <222> (1)..(7)  
 <223> SV40 large T antigen nuclear localization sequence  
 (NLS)

<400> 35  
 Pro Lys Lys Lys Arg Lys Val  
     1                    5

<210> 36  
 <211> 61  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:segment from  
       EcoRI to KpnI containing Kozak sequence including  
       initiation codon and SV40 NLS

<400> 36  
 gaattcgcta gcgccaccat ggcccccaag aagaagagga aggtgggaat ccattgggga 60  
 c 61

<210> 37  
 <211> 187  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:segment from  
       KpnI to XhoI containing BamHI site, KRAB-A box  
       from KOX1, FLAG epitope and HindIII site

<400> 37  
 ggtaccggg gatcccgac actggtgacc ttcaaggatg tatttgtgga cttcaccagg 60  
 gaggagtgga agctgctgga cactgctcag cagatcgtgt acagaaatgt gatgctggag 120  
 aactataaga acctggtttc cttgggcagc gactacaagg acgacgatga caagtaagct 180  
 tctcgag 187

<210> 38  
 <211> 277  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:inserted  
       fragment from BamHI to HindIII sites

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&lt;400&gt; 38

ggatccgccc ccccgaccga tgtcagcctg ggggacgagc tccacttaga cggcgaggac 60  
 gtggcgatgg cgcattgccga cgcgctagac gatttcgata tggacatgtt gggggacggg 120  
 gattccccgg ggccgggatt tccccccac gactccgccc cctacggcgc tctggatatg 180  
 gccgacttcg agtttgagca gatgtttacc gatgcccttg gaattgacga gtacggtggg 240  
 ggcagcgact acaaggacga cgatgacaag taagctt 277

&lt;210&gt; 39

&lt;211&gt; 118

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:sequence  
 replacing NLS-KRAB-FLAG with NLS-FLAG only

&lt;400&gt; 39

gaattcgcta gcgccaccat ggcccccaag aagaagagga aggtgggaat ccatggggta 60  
 cccgggggatg gatccggcag cgactacaag gacgacgatg acaagtaagc ttctcgag 118

&lt;210&gt; 40

&lt;211&gt; 204

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:insert into  
 MluI/BglII sites of pGL3-Control to create  
 pVFR1-4x

&lt;400&gt; 40

acgcgtaagc ttgctagcga gcggggagga tcgcggaggc ttggggcagc cgggtagagc 60  
 gagcggggag gatcgcgag gcttggggca gccgggtaga gcgagcgggg aggatcgagg 120  
 aggcttgggg cagccgggta gagcgagcgg ggaggatcgc ggaggcttgg ggcagccggg 180  
 tagagcgctc agaagcttag atct 204

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